

Name: \_\_\_\_\_

## 2007 7.013 Problem Set 5

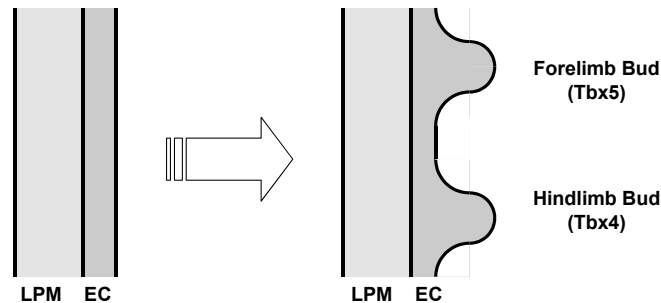
Due before 5 PM on WEDNESDAY, April 18, 2007.

Turn answers in to the box outside of 68-120.

PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

### Question 1.

The limb (arm or leg) forms when a group of cells called “lateral plate mesoderm” (LPM) induces overlying ectoderm (EC) to form an outgrowth called the “limb bud”.



The limb bud forms an outgrowth because the number of cells it contains increases relative to surrounding ectoderm.

- 1a. One possibility is that cells in the bud undergo increased cell division relative to surrounding ectoderm. What technique could you use to ask whether this possibility is correct? (5 words or fewer)

**BrdU labeling, which labels newly synthesized DNA. Or some other method that would mark dividing cells.**

- 1b. Give another possible mechanism that would increase cell number. (5 words or fewer)

**Decreased apoptosis in the limb bud, or increased apoptosis in the surrounding ectoderm.**

- 1c. Interestingly, part of the brain (the “hindbrain”) can substitute for the LPM and induce limb bud outgrowth. Discuss the molecular biology that is likely to explain this result (15 words or fewer).

**The hindbrain and the LPM both produce some factor (maybe the same one) that is required for limb bud outgrowth (and hindbrain development).**

- 1d. Young lateral ectoderm can give rise to many fates, including the limbs, brain and spinal cord, the epidermis of the skin, pigment cells. However, in older embryos, the lateral ectoderm can form only a limb bud. What is the term for the possible fates that a cell can form?

**Potency or potential**

Name: \_\_\_\_\_

The front and back legs of the chicken are very different, where the front limb is covered with feathers, and the hindlimb is covered with scales. In order to figure out why these limbs are different from one another, the following transplant experiment was performed. LPM from the hindlimb area was placed under future forelimb ectoderm, and LPM from the forelimb region was placed under future hindlimb ectoderm. The resulting chick embryos were assayed after the limbs had differentiated, and the following results were obtained.

future forelimb LPM	+	future forelimb ectoderm	→→	feathers
future hindlimb LPM	+	future forelimb ectoderm	→→	scales
future hindlimb LPM	+	future hindlimb ectoderm	→→	scales
future forelimb LPM	+	future hindlimb ectoderm	→→	feathers

- 1e. What do these data tell you regarding the mechanism by which fore- and hindlimbs make feathers or scales? (15 words or fewer)

**Feathers or scales are induced by the LPM, not by the ectoderm.**

Chickens have white feathers, quail have black feathers. You perform a transplant experiment like the one above, as detailed below, with the following results:

Chick forelimb LPM	+	chick ecto	→ → →	white feathers
Quail forelimb LPM	+	chick ecto	→ → →	white feathers
Chick forelimb LPM	+	quail ecto	→ → →	black feathers
Quail forelimb LPM	+	quail ecto	→ → →	black feathers

- 1f. What do these data tell you regarding the mechanism by which chick and quail make different color feathers? (15 words or fewer)

**Feather color is dependent on the ectoderm, not on the LPM.**

Name: \_\_\_\_\_

The Tbx4 transcription factor is expressed exclusively in the developing hindlimb, and is necessary for development of hindlimb characteristics, including whether it has scales or feathers. A mutant has been isolated where the hindlimb now looks like a forelimb.

Linkage analysis indicates that this mutant lies in the Tbx4 gene. However, in order to confirm mutant gene identity, you sequence the Tbx4 genomic DNA from this mutant, and find that there are no changes in any exon. On the other hand, you find two nucleotides in the mutant DNA, which differ from wild type. The first is at the exon1/intron1 splice acceptor sequence. The second is 25 bp 5' to the transcriptional start site, where the wild type sequence 5' TATAAAAT is instead 5'TATAGGGC.

- 1g. In the splice acceptor mutant, what changes would you observe in the RNA made from this gene, relative to wild type? (15 words or fewer)

**The first intron would not be spliced out.** This would result in a number of incorrect amino acids being added after exon 1, and probably an early stop codon or a frame shift.

- 1h. Could this change account for the Tbx4 mutant phenotype? Explain in 15 words or fewer.

**Yes. There would be no functional Tbx4 translated from the aberrant RNA transcript.**

- 1i. In the mutant that maps to the non-transcribed part of the gene (TATAAAAT), what changes would you observe in the RNA made from this gene, relative to wild type? (15 words or fewer).

**TATA binding factor would not bind to the Tbx4 promoter, so RNA would not be transcribed.**

- 1j. Could this change account for the mutant phenotype? Explain in 15 words or fewer.

**Yes. There would be no Tbx4 transcript produced, and therefore no protein.**

- 1k. Tbx5 is required for forelimb development, and normally expressed only in the forelimb. In the Tbx4 mutant, you find that Tbx5 is now expressed in both sets of limbs. Explain (use a diagram if you like).

**Tbx4 --| Tbx5 → forelimb characteristics**

**Tbx5 promotes forelimb characteristics. Tbx4 inhibits Tbx5.** With no Tbx4 in the mutant hindlimbs, Tbx5 is expressed there, resulting in forelimb characteristics.

Name: \_\_\_\_\_

## Question 2.

**2a.** Tube formation is often accompanied by lengthening or extension of an initially short tube. What two processes lengthen a cell sheet? Explain what each entails (15 words or fewer).

- (i) **Epiboly: The cells of the tube lengthen or stretch out.**
- (ii) **Convergent extension: Cells intercalate into the epithelial sheet forming the tube.**

The neural tube forms the brain and spinal cord. Wnt signaling is required for lengthening the tube. The future neural tube lies next to the notochord and the somites.

**2b.** Give two ways you could determine where the Wnt signal comes from? Your answers should be independent, and include...

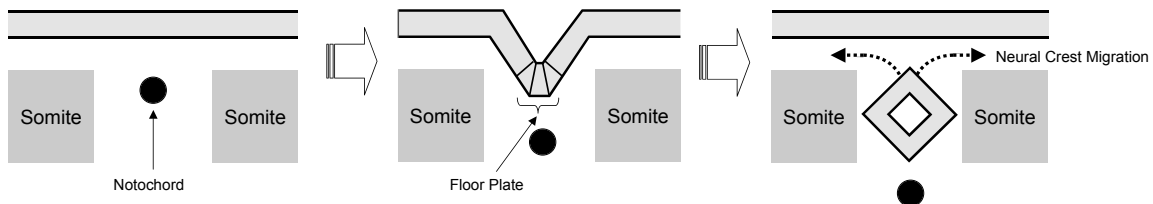
- (i) use of an explant assay

**Three explants: neural tube alone, neural tube + notochord, neural tube + somites. Determine which explant results in a lengthened neural tube. Whichever explant results in a lengthened neural tube contains the source of the Wnt signal.**

- (ii) use of gene expression assays

**In situ hybridization to determine which part of the embryo expresses Wnt RNA, or an antibody to detect Wnt protein (or Wnt-GFP).**

Formation of the neural tube involves a sharp ventral bend as diagrammed. This bend is associated with formation of wedge-shaped cells to form the “floorplate”.



**2c.** One gene which is expressed in the floorplate is “sonic hedgehog” (shh). How could you determine whether shh is necessary for floorplate formation? (15 words or fewer)

**Knock out the shh gene and see whether the floorplate forms, or add a shh inhibitor.**

**2d.** What structural cytoskeletal protein would you expect to change in the floorplate cells as they become wedge-shaped?

**Actin**

Name: \_\_\_\_\_

- 2e. How would this protein change?  
**See more F-actin. Either less f-actin and more g-actin at the narrower side of the cell, or more f-actin and less g-actin at the wider side.**
- 2f. The compound “blebbistatin” inhibits myosin function. If you applied blebbistatin to the developing floorplate, what effect would you expect to observe? Explain in 15 words or fewer.  
**There would be no wedge cell formation. Blebbistatin inhibits myosin function which is required for contraction of actin filaments.**
- 2g. As shown in the above diagram, the floorplate lies above a structure called the “notochord”. shh is also expressed in the notochord, and is responsible for inducing expression of shh in the floorplate. How does shh expression in the notochord activate shh expression in the floorplate? (15 words or fewer)  
**shh secreted by the notochord induces the floorplate to express shh via cell signaling.**

As diagrammed above, from the dorsal neural tube, an important group of cells called the “neural crest” begins to migrate to many parts of the embryo. When they reach their final destinations, they differentiate into pigment cells, the bones and muscles of the face, the peripheral nerves and part of the adrenal gland.

- 2h. Are the migratory neural crest cells likely to be single cells or a sheet of cells?  
**Single cells. Migratory cells are generally mesenchymal cells, which are single cells.**
- 2i. What cell type transition do the neural crest cells undergo as they leave the neural tube to become migratory?  
**Epithelial to mesenchymal transition.**

The protein collagen is the most abundant in the human body. It is crucial part of the extracellular matrix. Mutants with no collagen die very soon after fertilization, but those with decreased collagen function survive longer, but show no neural crest migration.

- 2j. Name one function of the extracellular matrix is important for cell migration? (15 words or fewer).  
**Substrate on which cell migrates. Adhesion. The cell forms focal adhesions with the ECM allowing f-actin polymerization inside the cell. ECM provides structure, something for the cell to grab onto.**

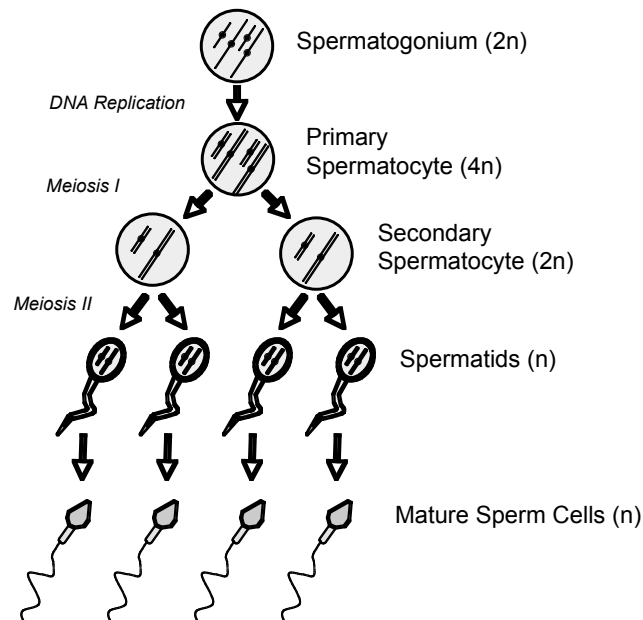
Neural crest cells that arrive at similar destinations stick to one another and go on to differentiate. Future adrenal medulla cells, and future jaw cells, can be dissociated into single cells by calcium removal, after they reach their destinations, but prior to differentiation. Addition of calcium will cause reassociation.

- 2k. After mixing the two dissociated populations together in the presence of calcium, what would you predict would happen? Include the term for this process. Explain in 15 words or fewer.  
**Cells will reassociate with their own kind in a process called “homotypic adhesion.”**

Name: \_\_\_\_\_

### Question 3.

The search for pluripotent stem cells is intense. Spermatogonial cells are the diploid precursors of haploid sperm according to the following scheme.



- 3a.** In order to ask whether mouse spermatogonia are stem cells, they were isolated from a donor and introduced into the testis of a host male of the sterile1 strain, where the testis looks normal, but lacks germ cells. The mouse recovered fertility. If the donor cells were from a mouse with black fur (B/B), and the host had white fur (b/b), devise a genetic cross to confirm that restoration of fertility was due to the donor B/B spermatogonia, and not to some other recovery of fertility by the b/b mouse. Assume b is recessive to B.

**bb male/transplanted with BB spermatogonia x bb female >> progeny should have black fur.**

- 3b.** You wonder whether other stages of sperm have stem cell capacity, and find that secondary spermatocytes do, but only for a few weeks after injection into the sterile1 strain. In contrast, the spermatogonia restore fertility for the rest of the sterile1 mouse' life. What property of a stem cell is missing in the secondary spermatocytes? (5 words or fewer)

**Self renewal.** The secondary spermatocytes are unable to divide for a long time to produce more stem cells.

The Sertoli cells normally surround the spermatogonial cells. In the sterile2 strain, there are no Sertoli cells, nor any germ cells.

- 3c.** If Sertoli cells normally comprise the “niche” of spermatogonial cells, would you predict that wild type donor spermatogonia introduced into the testes of the sterile2 strain would differentiate into sperm? Explain (15 words or fewer).

Name: \_\_\_\_\_

**No. The niche is necessary to signal to the stem cells.** Spermatogenesis requires signals from Sertoli cells.

- 3d. What is the relationship between cell-cell signaling, induction and the niche? (15 words or fewer).

**Cell-cell signaling (induction) from the niche cells induces stem cells.**

You also wonder whether what the potency the spermatogonial cells possess. You therefore make a transgenic animal, ROSA52, which specifically expresses the lacZ gene in the spermatogonia, which makes all cells in the animal blue, after appropriate staining. You then perform various repopulation experiments to test spermatogonial potency.

- 3e. What is the point of using blue cells?

**To determine whether they are host or donor cells.** This way, you can see which animal the cells came from.

You purify spermatogonial cells from a ROSA52 mouse and introduce them into an unlabelled lethally irradiated mouse.

- 3f. What is the point of the lethal irradiation?

**To kill the host cells so that you can test whether the spermatogonial cells can rescue the host.**

- 3g. You find that all blood and immune cells are blue in the repopulated mouse. What would you conclude regarding spermatogonial potency?

**Spermatogonia are capable of repopulating blood and immune cells as well as spermatogonial cells. They have high potency.**

Name: \_\_\_\_\_

#### Question 4.

4a. What are the two meanings of “cloning” to which you have been exposed in this course?

(i) **Making many copies of DNA using vectors in bacteria.**

(ii) **Making copies of whole organisms.**

The challenge of neuropsychiatry is to understand whether a disorder is caused by genetic mutation, or by other means. Identical twin studies are useful in this regard.

4b. What is the definition of “concordance” in this context? (5 words or fewer)

**A measure of shared traits. How frequently a trait is shared.**

4c. Studies on autistic disorder show a concordance of 0-23% for siblings, and 90% for monozygotic twins. What does this data indicate? (15 words or fewer)

**Autistic disorder has a strong genetic component.**

4d. In contrast, most forms of schizophrenia show a concordance of less than 50% in monozygotic male twins. What does this data indicate? (15 words or fewer)

**There is a genetic predisposition for schizophrenia, but something other than genetics contributes; there is a strong epigenetic component**

4e. A mouse model for schizophrenia has been developed, by disruption of a gene called DISC1, implicated in the human disorder. As in humans, only about 50% of the mice (which are a genetically identical homogenous strain) develop the disorder. You treat the mice with 5-azacytidine, a cytosine analog that cannot be methylated. You now find that 100% of the mice develop the disorder. Explain this result (15 words or fewer).

**5-azacytidine treatment leads to demethylation and activation of genes that promote the DISC1 phenotype.**

Embryos derived from fathers with loss of function in the (Insulin-like growth factor) IGF1 gene are very small. Embryos derived from mothers with loss of IGF1 function are of normal size.

4f. Of what is this phenomenon an example? (1 word) **Imprinting**

Mice derived by somatic cell nuclear transfer usually suffer from “large offspring syndrome”, where the neonates are several times larger than normal neonates.

4g. What prediction would you make regarding IGF1 protein expression in these embryos derived from SCNT? (5 words or fewer)

**Since some embryos that lack IGF1 are small, IGF1 is probably needed to make embryos bigger. These animals are too big and therefore embryos derived from SCNT probably express too much IGF1.**